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SHORT COMMUNICATION

Effect of the human endotoxin challenge on tedizolid tissue penetration

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The effects of the human endotoxin challenge on tissue pharmacokinetics are unknown. In the present study, we aimed to assess the effect of the endotoxin challenge on interstitial fluid pharmacokinetics of tedizolid in healthy volunteers using intramuscular microdialysis. Eight healthy male subjects were treated with 200 mg of tedizolid phosphate for 6 days. On Day 6, an intravenous bolus of lipopolysaccharide (LPS) (2 ng/kg body weight) was administered. LPS infusion did not affect plasma pharmacokinetics of tedizolid. In contrast, following LPS infusion, median muscle tissue *f*AUC (0.83 [0.75–1.15] *vs.* 1.14 [1.11–1.43] mg × h/L, $P = .0078$) and muscle tissue fC_{max} (0.15 [0.14–0.19] *vs.* 0.19 [0.18–0.24] mg/L, $P = .0078$) were significantly increased by 38% and 24%, respectively. The human endotoxin challenge was associated with increased tissue concentrations of tedizolid, without affecting its plasma concentration–time profile. The human endotoxin challenge combined with microdialysis may be used to investigate the influence of systemic inflammation on tissue pharmacokinetics.

KEYWORDS
antibiotics, antimicrobials, drug distribution, endotoxaemia, inflammation, lipopolysaccharide (LPS), microdialysis, pharmacokinetics

1 | INTRODUCTION

Tissue pharmacokinetics vary substantially between healthy volunteers and patients with inflammatory conditions or diseases.[1] Antibiotic pharmacokinetics are usually studied in healthy volunteers. In clinical practice, antibiotic use often coincides with infection-related systemic or local inflammation, which may affect blood and tissue pharmacokinetics of antimicrobials.[2] This may be important for antimicrobial therapies, whose success is highly dependent on their delivery to the target site (i.e., the site of infection). Because pharmacokinetic studies can impose a significant burden on infected patients, a disease model that facilitates the study of the effects of systemic inflammation on tissue pharmacokinetics would be desirable. The human endotoxin challenge is an established model of inflammation and has been used extensively to investigate many aspects of systemic inflammatory processes. However, it is not commonly used for tissue pharmacokinetic studies.[3,4]

Tedizolid, a second-generation oxazolidinone, is indicated for the treatment of skin and skin structure infections. Therefore, it is worthwhile to investigate the effects of systemic inflammation on the distribution of tedizolid in soft tissue. In the present study, intramuscular microdialysis was used to access a soft tissue compartment. We aimed to assess the effect of the endotoxin challenge on interstitial fluid (ISF) pharmacokinetics of tedizolid in muscle tissue in healthy volunteers.

2 | METHODS

We previously reported that tedizolid failed to mitigate the lipopolysaccharide (LPS)-induced cytokine response in healthy subjects.[5] In a sub-study in 8 of the 14 included subjects, we additionally performed intramuscular microdialysis to assess the effect of LPS infusion on the ISF concentrations of tedizolid. We included male subjects aged 18–55 who had an unremarkable medical history and physical examination. Subjects with a body weight below 60 or over 95 kg, smokers and subjects with allergies to substances used in this study were excluded.

In brief, each subject received tedizolid phosphate 200 mg (Sivextro®, Merck Sharp & Dohme Corp, Kenilworth, NJ, United States) orally once daily for 3 days, followed by 3 days of tedizolid phosphate 200 mg intravenously over 60 min once daily. This dosing regimen is recommended for the treatment of skin infections according to the package leaflet. Considering the high oral bioavailability (over 90%) and the half-life of about 12 h, steady-state pharmacokinetics were reached no later than on Day 3. Blood and intramuscular microdialysis sampling were performed under steady-state conditions on Day 5 (control day) and on Day 6 (LPS day). On Day 6 (i.e., the last day of treatment), the endotoxin challenge was performed at the same time as the last tedizolid infusion. Each subject received 2 ng/kg body weight LPS (*Escherichia coli* O113 Reference Endotoxin, CC-RE Lot 3) intravenously over 1–2 min followed by an 8-h infusion of 0.9% saline solution at an infusion rate of 100 mL/h. In addition, 1 g of paracetamol (Paracetamol Genericon® 500 mg, Genericon Pharma GmbH, Graz, Austria) was given orally to prevent and relieve endotoxin-associated symptoms.

Microdialysis was performed as previously described.[6] On the morning of Day 5, an intramuscular microdialysis probe (membrane length: 10 mm; molecular weight cut-off: 20 kDa) was inserted into thigh muscles and constantly perfused with 0.9% saline at 1 μL/min for 8 h. This probe remained in the same location throughout the sampling period on Days 5 and 6. Since relative recovery never reaches 100%, concentrations obtained by microdialysis must be corrected by a factor that is determined by retrodialysis. In brief, the perfusion solution containing a known concentration of the tedizolid was pumped through the microdialysis system. By measuring the tedizolid concentration in the dialysate fluid, the relative loss, which equals the relative recovery, can be calculated (recovery [%] = 100 − [$\text{concentration}_{\text{dialysate}}$/$\text{concentration}_{\text{perfusate}}$ × 100]).[7] These retrodialysis experiments were performed following the 8-h sampling phase on both sampling days (Days 5 and 6). Relative recovery calculation and dialysate concentration correction were performed as previously described.[6] Tedizolid concentrations were determined by high-performance liquid chromatography-ultraviolet (HPLC-UV), and plasma protein binding by ultrafiltration as previously described.[8] We performed a non-compartmental analysis to calculate pharmacokinetics of tedizolid in tissue. Results are reported as median with interquartile range (IQR). Statistical testing between the control and LPS day was done using the non-parametric Wilcoxon matched-pairs signed-rank test. Because of the exploratory nature of this study, no formal sample size calculation was performed.

What is already known about this subject

- Tissue pharmacokinetics vary substantially between healthy volunteers and patients with inflammatory conditions or diseases.
- The human endotoxin challenge has been used extensively to investigate many aspects of systemic inflammation but rarely for pharmacokinetic studies.

What this study adds

- The endotoxin challenge was associated with increased muscle tissue concentrations of tedizolid, without affecting its plasma concentration–time profile.
- The human endotoxin challenge combined with microdialysis may be a promising tool to evaluate the effect of systemic inflammation on target site pharmacokinetics.

This project was conducted following the International Council for Harmonisation-Good Clinical Practice (ICH-GCP) guidelines and the Declaration of Helsinki. All study participants were informed and gave their oral and written consent prior to inclusion. The study was registered at the EudraCT database (EudraCT 2018-004743-23). It was approved by the Ethics Committee of the Medical University of Vienna (EC 2251/2018) and the Austrian Agency for Health and Food Safety.

2.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries (in www.guidetopharmacology.org) and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.

3 | RESULTS

A total of eight healthy male subjects underwent plasma and microdialysis sampling on Days 5 and 6 of treatment with tedizolid 200 mg once daily. The median (IQR) age was 26 (24–29.5) years, and the median (IQR) body mass index was 24.2 (21.5–26.5) kg/m^2. Tedizolid treatment was well tolerated. In a recent article examining the anti-inflammatory effects of tedizolid, we described the clinical symptoms and cytokine release during endotoxaemia in these participants.[5] The reactions were as expected, including a pronounced cytokine response and flu-like symptoms.[5] Figure 1 shows the free plasma and muscle ISF concentration−time curves of tedizolid on the control day (Day 5) and the day of LPS infusion (Day 6). While the plasma

concentration profiles were similar between the two days, the tissue concentrations were significantly higher on the day of the endotoxin challenge, especially in the initial 4 h. In plasma, the free 8-h area under the curve ($fAUC_{0-8}$) (median [IQR]; 2.6 [2.2–2.8] *vs.* 2.5 [2.4–3.0] mg × h/L; $P = .64$), free maximum concentration (fC_{max}) (0.61 [0.50–0.71] *vs.* 0.57 [0.55–0.63] mg/L; $P = .74$), and protein binding (77.6% [76.7–78.2] *vs.* 77.7% [76.4–78.4]; $P = .38$) were similar between the control and LPS day (Figure 2A–C). In contrast, following LPS infusion, median muscle ISF *f*AUC (0.83 [0.75–1.15] *vs.* 1.14 [1.11–1.43] mg × h/L, $P = .0078$) and muscle ISF fC_{max} (0.15 [0.14–0.19] *vs.* 0.19 [0.18–0.24] mg/L, $P = .0078$) were significantly increased by 38% and 24%, respectively (Figure 2D,E). This increase was observed in each of the eight study participants. The median tissue half-life of tedizolid over Days 5 and 6 was 14.8 h (IQR 11.7–18.8), indicating that steady-state tissue pharmacokinetics (or five half-lives) were achieved after a median of 3.1 days (IQR 2.4–3.9). After excluding the only subject with a significantly longer half-life (85 h) from the analysis, the difference in ISF C_{max} and AUC between the two study days remained significant. The relative recovery rates calculated from retrodialysis were constant over the two sampling days and independent of LPS infusion (control *vs.* LPS: 85.8% [82.6–87.5] *vs.* 83.1% [81.1–87.0], $P = .84$) (Figure 2F).

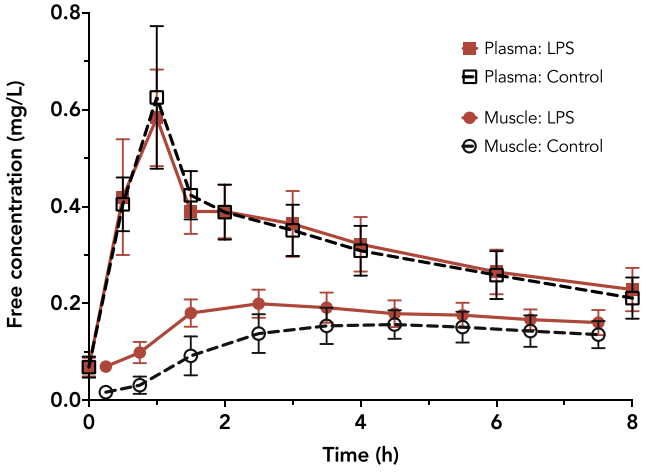

FIGURE 1 Free tedizolid concentration–time profiles in plasma and in muscle interstitial fluid as determined by microdialysis on the day before ('Control') and the day of the endotoxin challenge ('LPS'). Concentrations are mean ± standard deviation. Endotoxin and tedizolid were administered together at time point 0. The dots showing intramuscular concentrations represent the mean concentration at the midpoint of the sampling interval (i.e., the concentration of the sampling interval between 0 and 30 min is depicted by a dot at 15 min)

4 | DISCUSSION

In this microdialysis study, the human endotoxin challenge was associated with unchanged plasma pharmacokinetics but increased muscle ISF penetration of tedizolid. The difference in muscle ISF

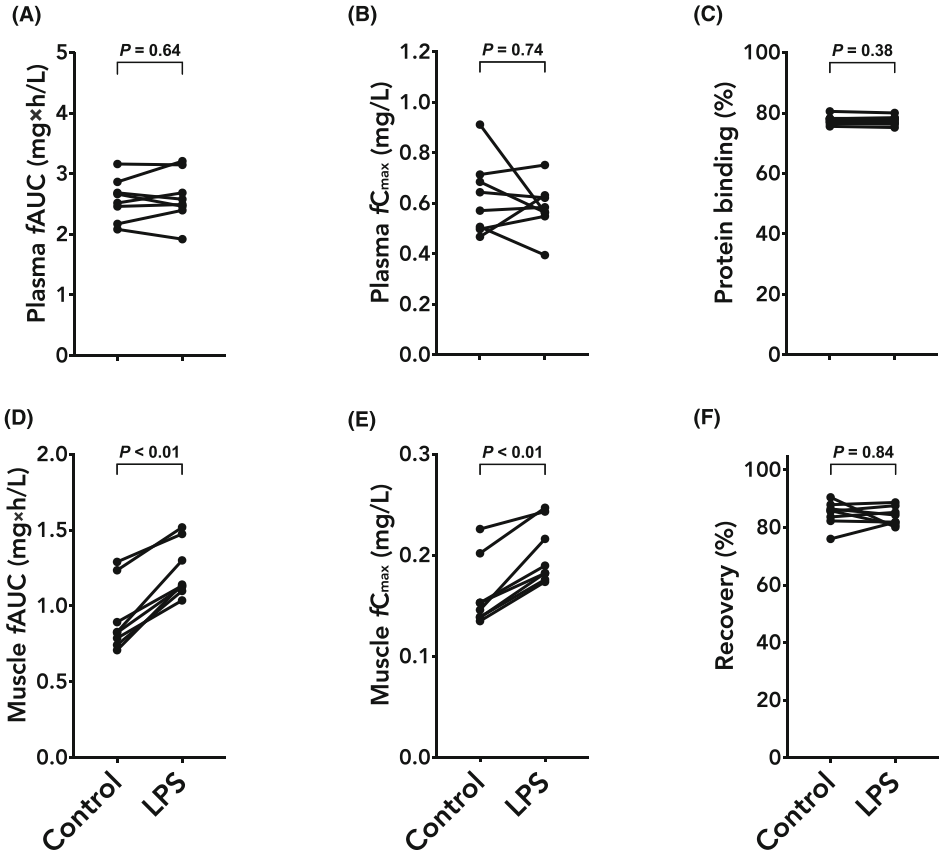

FIGURE 2 Comparisons of the plasma and microdialysis results between the day before *vs.* the day of the endotoxin challenge ('Control' *vs.* 'LPS'). (A, B) Free 8-h area under the curve ($fAUC_{0-8}$) and free maximum concentration (fC_{max}) in plasma. (C) Plasma protein binding (%) assessed by ultrafiltration. (D, E) *f*AUC and fC_{max} in muscle interstitial fluid assessed by microdialysis. (F) Relative recovery rates (%) assessed by retrodialysis

concentration was especially evident in the initial 4 h after LPS infusion and seemed to diminish from then on, consistent with the clinical and biochemical response to LPS.[5,9] In general, the tedizolid tissue concentrations from our study were comparable with those observed by Stainton et al.[10] but lower than those observed by Sahre et al.[11] After five half-lives, it can be assumed that steady-state pharmacokinetics are reached (or at least 97%).[12] This means that a dynamic equilibrium exists and that further doses do not lead to further accumulation. Our data suggests that this steady state was reached after 3 days in the tissue of most participants and that there was no further accumulation between Days 5 and 6.

Stainton et al.[10] compared tedizolid tissue concentrations between healthy volunteers and patients with diabetic foot infections using microdialysis. Compared with healthy volunteers, tissue-to-plasma ratios of tedizolid were numerically higher in patients with diabetic foot infections, without reaching statistical significance.[10] Another microdialysis trial in patients with diabetic infections showed higher concentrations of ertapenem in inflamed tissue.[13] In contrast, increased penetration was not shown for fosfomycin and ciprofloxacin in similar studies.[14,15] It is unclear why increased penetration was only observed for some of the antibiotics studied. Notably, diabetic infections differ from the setting investigated in our study. Although diabetic infections cause an inflammatory response, they are also characterized by impaired blood perfusion, which could reduce drug penetration into tissue.[16] These two opposing effects may affect different compounds to varying degrees. Therefore, the results in diabetic foot infections must be compared with ours with caution.

The exact mechanisms causing the enhanced penetration of tedizolid into muscle ISF remain unclear. It could be speculated that an LPS-induced increase in tissue perfusion and vascular permeability might have played a role. TNF-α, a cytokine that is released during endotoxaemia, caused vasodilation of vessels that were pre-treated with LPS.[17] Wellhoener et al.[18] found evidence of a greater than two-fold increase in adipose tissue blood perfusion after LPS infusion in healthy volunteers. In addition, TNF-α was shown to increase vascular permeability by interacting with the transient receptor potential channel.[19] LPS administration increased vascular permeability in vitro[20] and in animal studies[21]; an endotoxin challenge trial including healthy volunteers, however, failed to confirm increased vascular permeability.[22]

Our study has several limitations. First, only the inclusion of an additional control group could have confirmed that differences in tissue concentrations were caused only by LPS and not by other contributing factors. Second, the control day was always the day before the day of the endotoxin challenge, which may have introduced a potential bias. Randomization between the two study days would not have been possible since the effects of the endotoxin challenge may persist for several months. The subjects received paracetamol on Day 6 but not Day 5. Although a possible interaction between paracetamol and tedizolid pharmacokinetics cannot be ruled out, we found no evidence of such an interaction in the literature. Administration of paracetamol the day before the endotoxin challenge or not administering paracetamol to symptomatic participants during endotoxaemia could have solved this problem. However, neither would have been justified in our opinion. However, the two sampling days were under steady-state conditions on Days 5 and 6 of the tedizolid treatment. The constant plasma pharmacokinetics support the comparability of both sampling days. Third, the results rely on consistent performance of the microdialysis system between Days 5 and 6. Simmel et al.[23] performed a proof-of-principal study and showed that microdialysis catheters can be used for 4 days. Kirbs et al.[24] also used microdialysis reliably for 80 h, supporting the long-term stability of microdialysis over several days. In addition, relative recovery was similar between the control and the LPS day, confirming similar diffusion across the microdialysis membrane on the two sampling days. Fourth, we studied only one compound, and it is unclear whether our results are generalizable to other antibiotics. We speculate that antibiotics with similar pharmacokinetic profiles (i.e., lipophilic and high volume of distribution) might exhibit a similar increase in tissue penetration after LPS administration.

Our findings warrant further similar studies to confirm this for other antibiotics. If future research can reproduce the effect of LPS on tissue penetration of other antimicrobial agents, the human endotoxin challenge could be used in combination with microdialysis to identify antibiotics whose tissue pharmacokinetics are more or less likely to be affected by systemic inflammation.

5 | CONCLUSIONS

To the best of our knowledge, this is the first human endotoxin challenge study investigating the effect of LPS on tissue pharmacokinetics. Intravenous administration of LPS appears to enhance the penetration of tedizolid into muscle ISF without affecting its plasma concentration–time profile. Although this observation was consistent across all eight subjects, the lack of a control group makes it impossible to conclusively infer the causal relationship between endotoxin exposure and increased tedizolid tissue concentrations. Moreover, it is unclear whether the delivery-enhancing effects of LPS are universal or specific to particular drugs or drug classes. Considering our findings, the human endotoxin challenge may be a promising tool to evaluate the effect of systemic inflammation on target site pharmacokinetics.

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COMPETING INTERESTS

The authors have no conflicts of interest to declare.

CONTRIBUTORS

B.W., C.S., P.M., B.J. and M.Z. prepared the study protocol. B.W., P.M., V.A.J., M.B., M.W.D. and E.L. performed the research. C.D. analysed the pharmacokinetic samples. A.J. and M.Z. analysed the data. A.J. wrote the first draft of this manuscript. All authors critically revised the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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